REMARKS

Status of the Claims

Claims 1-4 are pending in the present application. Claims 5-6 were previously canceled. Claim 1 is amended.

Support for claim 1 is found throughout the specification as originally filed. For example, support for "collecting human cartilage having perichondrium", is found on page 9, line 2, which specifies "[a] cartilage tissue is excised and disinfected...."

Support for, "treating the cartilage having perichondrium with type II collagenase" is found, *e.g.*, on page 8, lines 22-24, which specifies "an excised cartilage tissue is diced with a surgical knife or the like, treated with collagenase and then cultured and proliferated." Support is also found, *e.g.*, on page 9, lines 7-8, which specifies, "[t]he diced cartilage tissue is then transferred into a medium containing type II collagenase...."

Support for "centrifuging the treated cartilage having perichondrium in step 2) to obtain precipitate; and 4) culturing the precipitate of step 3)", is found, *e.g.*, on page 9, lines 10-11, which specifies "thus treated tissue is centrifuged and the obtained precipitate is employed in the culture."

Support for "wherein the chondrocytes and perichondrium are both in the culture" is found, *e.g.*, on page 4, lines 6-9, which specifies "[a] method of producing human chondrocytes characterized by comprising co-culturing chondrocytes obtained from a cartilage having perichondrium together with the perichondrium."

Support for "wherein no non-human animal feeder cells are present in step 4)" is found, *e.g.*, on page 6, lines 10-14, which specifies "the method according to the present invention can be conveniently carried out while omitting the troublesome procedures needed in the case of using feeder cells, and, moreover, the risk of infection from the feeder cells and so on can be avoided thereby."

Support for "further wherein the cells increase to at least 1 X 10^6 cells from 1 cm^2 tissue in primary culture and further increase at least 1000 times in subculture" is found on page 13, lines 15-16, which specifies "[s]ubculture was carried out by seeding 1 x 10^6 of the primary-cultured cells." Support is also found on, *e.g.*, page 13, lines 20-23, which specifies, "[t]he cell

count on the fourth subculture increased about 1000 times, compared with the cell count at the initiation of the subculture." Further support is found, *e.g.*, on page 10, lines 23-24, which specifies "[t]he cell count increases about 1000 times from P0 (primary culture) to P4."

Issues Under 35 U.S.C. § 112, First Paragraph

Claim 1 is rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement, *see* Office Action, page 2. Specifically, the Examiner asserts that the claim is confusing and introduces new matter.

Claim 1, as amended, is directed to a method of proliferating human chondrocytes comprising 1) collecting human cartilage having perichondrium; 2) treating the cartilage having perichondrium with type II collagenase; 3) centrifuging the treated cartilage having perichondrium in step 2) to obtain precipitate; and 4) culturing the precipitate of step 3), wherein the chondrocytes and perichondrium are both in the culture, and wherein no non-human animal feeder cells are present in step 4), and further wherein the cells increase to at least 1×10^6 cells from 1 cm^2 tissue in primary culture and further increase at least 1000 times in subculture.

Support for each element of amended claim 1 is supported by the instant application as described above. Accordingly, no new matter is introduced by amended claim 1. Further, claim 1, as amended, clearly describes Applicants' invention. Based upon the foregoing, Applicants respectfully request withdrawal of the rejection.

Issues Under 35 U.S.C. § 112, Second Paragraph

Claims 3 and 4 are rejected for specifying "cultured chondrocytes" due to insufficient antecedent basis, *see* Office Action, page 3. As amended, claim 1 specifies chondrocytes and perichondrium are both in the culture. Accordingly, the phrase "cultured chondroctyes" has antecedent basis. Based upon the foregoing, Applicants believe the rejection is overcome and respectfully request withdrawal of the rejection.

Issues Under 35 U.S.C. § 103(a)

Claims 1-4 are rejected under 35 U.S.C. § 103(a), as allegedly obvious over the combination of Megerian *et al.*, *Tissue Engineering*, 2000, 6:69-74, ("Megerian") and van Osch *et al.*, *Plast. Reconstr. Surg.*, 2001, 107:433-440 ("van Osch I") in view of Klein-Nulend *et al.*,

Tissue Engineering, 1998, 4:305-313, ("Klein-Nulend") and van Osch *et al.*, *Tissue Engineering*, 2000, 6:321-330 ("van Osch II"), as evidenced by Sucheston *et al.*, *Ohio J. of Science*, 1969, 69:366-370, ("Sucheston"). Specifically, the Examiner alleges that Megerian teaches a method of producing chondrocytes from auricular cartilage comprising collecting auricular cartilage, treating the cartilage with collagenase and culturing the chondrocytes without feeder cells, *see* Office Action, page 5. According to the Examiner, van Osch I teaches isolating human auricular cartilage and culturing the isolated chondrocytes in a monolayer for 3-4 passages without the use of exogenous feeder cells, *see* Office Action, page 5.

The Examiner admits that Megerian and van Osch I do not teach cartilage bonded to perichondrium, *see* Office Action, page 5. However, the Examiner states that Klein-Nulend teaches culturing human auricular perichondrium without the use of non-human feeder cells, *see* Office Action, page 5. Sucheston is cited to demonstrate that perichondrium contains chondrocytes, *see* Office Action, page 5. The Examiner further states that van Osch II teaches growing cartilage *in vitro* from auricular perichondrium, which differentiates into chondrocytes and can generate cartilage, *see* Office Action, page 6. According to the Examiner, it would have been obvious to an ordinary artisan to culture cartilage with perichondrium in a method of producing chrondrocytes because the perichondrium is a convenient source of cells with chondrogenic potential, *see* Office Action, page 6. The Examiner further states that an ordinary artisan would have been motivated to culture cartilage as described in Megerian or van Osch I with the perichondrium described in Klein-Nulend or van Osch II with a reasonable expectation of success since perichondrium from ear or rib is described by Klein-Nulend as a convenient source of cells with chondrogenic potential, *see* Office Action, page 6.

As noted above, independent claim 1, as amended, is directed to a method of proliferating human chondrocytes comprising 1) collecting human cartilage having perichondrium; 2) treating the cartilage having perichondrium with type II collagenase; 3) centrifuging the treated cartilage having perichondrium in step 2) to obtain precipitate; and 4) culturing the precipitate of step 3), wherein the chondrocytes and perichondrium are both in the culture, and wherein no non-human animal feeder cells are present in step 4), and further wherein the cells increase to at

least 1 X 10^6 cells from 1 cm^2 tissue in primary culture and further increase at least 1000 times in subculture.

In contrast to the instant claims, none of the cited references disclose culturing chondrocytes with perichondrium, as the Examiner indicates, *see* Office Action, pages 5-7. Further, because the chondrocytes are cultured with perichondrium, the cells increase into unexpected numbers, *i.e.*, at least 1 X 10^6 cells from 1 cm^2 tissue in primary culture, which further increases at least 1000 times in subculture. This unexpected advantage, which is specified in the instant claims, is not disclosed or suggested in the cited references.

The Examiner notes that Klein-Nulend teaches that the perichondrium from ear or rib is used as a convenient source of cells for obtaining chondrocytes. In contrast, the chondrocytes described in the instant claims are obtained from the cartilage having perichondrium, which is treated with type II collagenase. Accordingly, the major source of perichondrocytes of the present invention is considered to be a part of the cartilage, *i.e.*, not a part of the perichondrium. This difference between the instant claims and the cited references is reflected by the number of cells, which can be obtained from tissue. Klein-Nulend only obtained 100 chondroctyes from a mm^2 area, *i.e.*, 1 x 10^4 cells/ cm^2. In contrast, 1 x 10^6 cells are obtained from 1 cm^2 of tissue in the instantly claimed methods.

van Osch II also fails to describe to describe this increase in cell number. van Osch II describes a culture using perichondrium without cartilage, *see* page 322, 4th line from bottom. van Osch II obtained only 1 x 10^6 cells in the subculture. In contrast, the instant claims describe that 1 x 10^6 x 1000 cells can be obtained.

Megerian teaches that perichondrium is not included in the culture. At page 71, lines 5-6, Megerian state "squares of cartilage were dissected from the underlying perichondrium" in the open procedure. At page 71, line 11, Megerian state that the cells were corrected by "a 12-guage core biopsy needle", by which perichondrium cannot apparently be corrected. Although Megerian does state that "cells are cultured until confluence was reacted", the number of cells obtained is not described. Furthermore, Table 2 indicates that the doubling time for the cells is 5.6 days, at the shortest. This doubling time means that the cells proliferate only 32 times (=2^5) at most in 28 (5.6 x 5) days. In contrast, as described in the instant application, the cells

unexpectedly increase at least 1000 times in 28 days, *see* page 10 of the present application, which describes four subcultures, 7 days per subculture.

Based upon the foregoing, the claims are not obvious over the cited references. Accordingly, Applicants respectfully request withdrawal of the rejection.

CONCLUSION

In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: MAY 18 2009

Respectfully submitted,

By _____
Gerald M. Murphy, Jr.
Registration No.: 28,977
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road
Suite 100 East
P.O. Box 747
Falls Church, Virginia 22040-0747
(703) 205-8000
Attorney for Applicant